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Tubakia seoraksanensis, a new species from Korea

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Abstract — An unknown species of *Tubakia* was collected recently from *Quercus mongolica* on Seoraksan Mountain, GangWon Province, in Korea. This species was characterized with cultural, ITS region sequence, and morphological data. After comparison with known species of *Tubakia*, this species is here described as *Tubakia seoraksanensis*, sp. nov.

Key words — *Ascomycota*, *Diaporthales, Dicarpella,* forest pathogen, *Sordariomycetes*

Introduction

The anamorphic genus *Tubakia* B. Sutton (*Diaporthales*, *Ascomycota*) causes leaf spot diseases on *Castanea, Quercus,* and various other trees and shrubs primarily in the *Fagaceae* (Sinclair et al. 2005). Currently, five species are recognized in *Tubakia*, namely *T. castanopsidis* (T. Yokoy. & Tubaki) B. Sutton, *T. dryina* (Sacc.) B. Sutton, *T. japonica* (Sacc.) B. Sutton, *T. rubra* (T. Yokoy. & Tubaki) B. Sutton, and *T. subglobosa* (T. Yokoy. & Tubaki) B. Sutton (Belisario 1991, Limder & Cash 1945, Sutton 1973, Yokoyama & Tubaki 1971). *Tubakia* has been associated with the teleomorphic genus *Dicarpella* Syd. & P. Syd. with the single species *D. dryina* Belisario & M.E. Barr connected to *T. dryina* (Belisario 1991).

In Korea, three species of *Tubakia* have been reported: *T. japonica* on *Castanea crenata* Siebold & Zucc., *Quercus acutissima* Carruth., and *Q. aliena* Blume; *T. rubra* on *Q. serrata* Murray; and an undescribed *Tubakia* sp. on *Q. rubra* L. (Cho et al. 2004, Lee et al. 1991,). In August 2009, specimens of *Tubakia* were collected on leaves of *Quercus mongolica* Fisch. ex Ledeb. (Mongolian oak) in Seoraksan Mountain, Gangwon Province, Korea. *Quercus mongolica* is a mountain tree that grows on ridgelines in Korea and is resistant to drought and wind conditions (Lee 1997). We determined that the specimens represented a previously undescribed species of *Tubakia*, which we then

analyzed using cultural, molecular, and morphological methods and compared with known species. The new species is described below.

Materials & methods

Fungal Isolations

Diseased leaves of *Quercus mongolica* were collected from nature. Isolates from fructifications were made from two different areas on the leaves, namely necrotic areas surrounding veins and leaf spots. Cultures were grown on malt extract agar (MEA, 1.5% malt extract and 2.0% agar) at 25°C in the dark. Specimens were deposited at the U.S. National Fungus Collections, Beltsville, Maryland, USA (BPI 880798 and BPI 880799) and cultures were deposited in CBS Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS 127490, CBS 127491, CBS 127492, and CBS 127493).

Morphological examination

Observations of microscopic structures were made from material mounted in distilled water and measured using a compound light microscope (Axiophot, Zeiss, Germany). Colors of cultures were described using Munsell soil color charts (Macbeth 2000).

DNA extraction, PCR, sequencing, analysis of sequence data

DNA was isolated from cultures on MYEA (2% malt extract, 0.2% yeast extract, and 1.5% agar) grown for about 10 to 14 days at room temperature. Mycelium was used for DNA extraction using PrepMan™ Ultra reagent following the manufacturer's instructions (Applied Biosystems, Foster City, CA). The internal transcribed spacer regions 1 and 2 and the 5.8 S rDNA hereafter referred to as ITS were amplified using the primer pair, ITS-1F and ITS4 (Gardes & Bruns 1993). Amplification of the ITS region was achieved with initial denaturation at 85°C 2 min; 35 cycles, each consisting of denaturation at 94°C for 1 min 35 sec, annealing at 49°C for 1 min, and extension at 72°C for 1 min; and final extension at 72°C for 15 min. PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. Automated sequencing was performed at the Iowa State University DNA Sequencing and Synthesis Facility (Ames, IA). Reactions were set up using Applied Biosytems (Foster City, CA) Prism BigDye Terminator v3.1 cycle sequencing kit with AmpliTaq DNA polymerase, FS and analyzed on an Applied Biosystems 3730 DNA analyzer.

Sequences were deposited at GenBank sequence database (HM991734, HM991735, HM991736, and HM991737). A GenBank Blast search was performed with the resulting sequence data and a comparison was made to existing *Tubakia* ITS region sequences. All sequences of *Tubakia* species were aligned and compared using the jPHYDIT program (Jeon et al. 2005).

Results: Analysis of sequence data & taxonomy

The ITS sequences from each of the four isolates were identical. The GenBank Blast search revealed the closest identifiable match as *Dicarpella dryina* (= *Tubakia dryina*, accession no. AY853242, culture CBS 115970) with 94% maximum identity. Additional

comparison with sequences of all other *Tubakia* species revealed a close relationship with *Tubakia japonica* (unpublished data). In the ITS region alignment produced in PHYDIT, sequences of *Tubakia seoraksanensis* were 98% similar to *T. japonica* and 97.8% similar to *D. dryina* (accession no. AY853242) (unpublished data).

Tubakia seoraksanensis H.Y. Yun, **sp. nov.** Fig. 1

MycoBank MB 519212

Coloniis in MEA similis Tubakiae japonicae *coloratis, sed conidiis minoribus, 13-25 µm × 10-15 µm, differt.*

Type: Korea, Gangwon, Seoraksan National Park, Seorak-dong, Sokcho-si, Gangwon-do, on living leaves of *Quercus mongolica,* 31.VIII.2009, coll. Hye Young Yun. **Holotype**, BPI 880799 (cultures CBS *127490*, ITS GenBank HM991734; CBS *127491*, ITS GenBank HM991735); **Isotype**, BPI 880798 (cultures CBS *127492,* ITS GenBank HM991736; CBS *127493*, ITS GenBank HM991737).

Etymology: referring to the type locality.

Leaf spots epiphyllous, occasionally amphigenous, 2–10 mm diam., globose to broadly ellipsoidal on blade, at times necrotic areas ellipsoidal to fusiform, extending along midrib or veins, pale brown, margin regular or irregular in shape, dark brown; typically discrete, may become confluent. Conidiomata epiphyllous, occasionally amphigenous, frequently but not always associated with spots, scattered to gregarious, sometimes confluent, especially near veins, superficial, easily removed from leaf surface, brown to dark brown, scutellate, each attached to leaf by a central columella, circular or somewhat irregularly subcircular as viewed from above, more or less plane as viewed from side. Scutella 90–160 × 90–130 µm, membranous, composed of thick-walled, pale brown to brown, 4–6 µm diam., septate hyphae radiating, typically bifurcating up to three times from a central disc that consists of a single hyaline cell towards margin, acute and cornute at margin that is fringed and unattached to substrate. Conidiogenous cells formed on underside of scutella, radiating downward and towards margin, 14–22 × 3–5 µm, cylindrical or slightly clavate to fusiform, narrowing to a thin point at neck, pale brown to hyaline. Conidia 13–25 × 10–15 µm (Q = 1.15–1.56), acrogenous, blastic, subglobose, broadly ellipsoid to ellipsoid, hyaline, becoming pale yellowish brown, walls smooth, thickening in age, with a prominent frill at base. Microconidia unknown.

In culture: Colony (after 10 days on MEA at 25°C in the dark) 32–44 mm diam., low velutinous to fuzzy, wrinkled, margin with uneven growth, whitish to pale yellow (8/1–2), reverse wrinkled, center darker, becoming progressively paler towards margin, olive brown, light olive brown to yellow (4/3, 5/6, 8/6), margin white (8/1), without sporulation. Mycelium branching, septate, 3.2–4.8 µm diam., hyaline or slightly brownish in mass, some hyphae forming short coils on side branches.

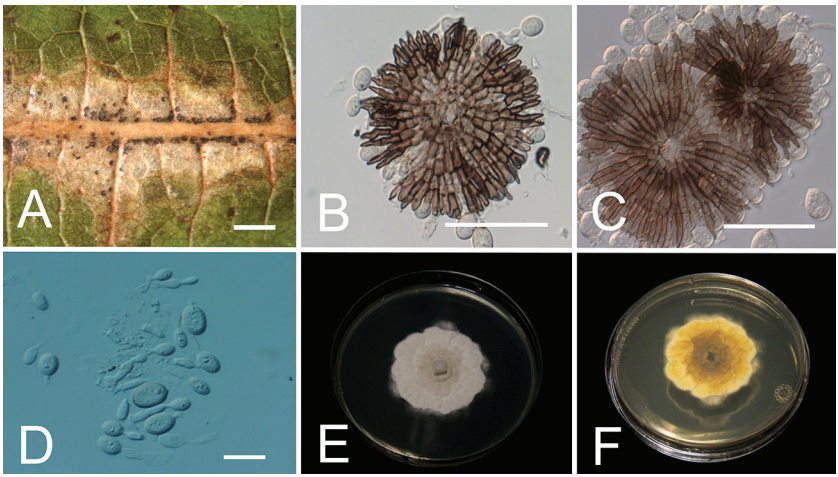

Fig. 1. *Tubakia seoraksanensis* (A–D, holotype; E–F, CBS 127491). A. Necrotic leaf spot and conidiomata along leaf midrib of *Quercus mongolica*. B. Scutellum and conidia by light microscopy (LM). C. Scutella and conidia by LM. D. Conidia and conidiophores by LM. E. Colony on MEA. F. Colony reverse on MEA. (Scale bars: A = 2mm, B–C = 50 µm, d = 20 µm)

Habitat & Distribution — Known only from *Quercus mongolica* at the type locality in Seoraksan National Park, Republic of Korea.

Comments — *Tubakia seoraksanensis* can be distinguished from all known species of *Tubakia* except *T. japonica* by its larger conidia. In culture on MEA, the colony color of *T. seoraksanensis* resembles that of *T. japonica* but is readily distinguished from the other species as illustrated in plate 2 of Yokoyama & Tubaki (1971).

Conidial size is the primary morphological difference distinguishing *T. japonica* and *T. seoraksanensis,* with *T. japonica* producing conidia that are much larger (40–55 × 35–45 µm) compared to those of *T. seoraksanensis* (13–25 × 10–15 µm). Yokoyama & Tubaki (1971) noted that the sizes of scutella, columellae, and conidia and hosts are useful in differentiating species. As with Jones & Holcomb (1978), Glawe & Crane (1987), and Taylor (2001), we found columellar characters untenable due to difficulty in observing these fragile structures and unreliable historical data.

Isolates of *T. seoraksanensis* from the different parts of the leaves (e.g., leaf blades or surrounding veins) produced identical sequences and conidiomatal morphology. However, necrotic areas surrounding veins tended to extend some distance along veins, be more irregular in shape, and were associated with a greater number of epiphyllous and hypophyllous conidiomata. The leaf spots on the leaf blades tended to be more confined in size, more regularly circular

in shape, and associated with a smaller number of epiphyllous and especially hypophyllous conidiomata.

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